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Mini-Review

Emerging Biomedical Applications of the Vesicular Stomatitis Virus Glycoprotein

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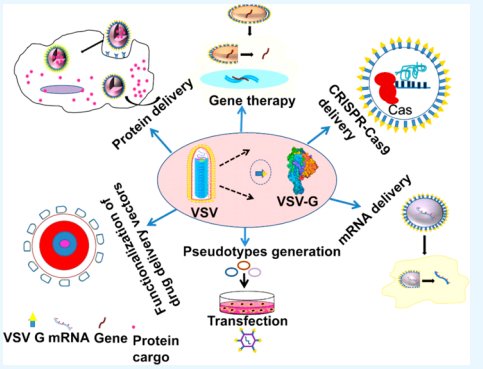
ABSTRACT: Nanoparticles (NPs) made of metals, polymers, micelles, and liposomes are increasingly being used in various biomedical applications. However, most of these NPs are hazardous for long- and short-term use and hence have restricted biomedical applications. Therefore, naturally derived, biocompatible, and biodegradable nanoconstructs are being explored for such applications. Inspired by the biology of viruses, researchers are exploring the viral proteins that hold considerable promise in biomedical applications. The viral proteins are highly stable and further amenable to suit specific biological applications. Among various viral proteins, vesicular stomatitis virus glycoprotein (VSV-G) has emerged as one of the most versatile platforms for biomedical applications. Starting with their first major use in lentivirus/retrovirus packaging systems, the VSV-G-based reagents have been tested for diverse biomedical use, many of which are at various stages of clinical trials. This manuscript discusses the recent advancements in the use of the VSV-G-based reagents in medical, biological research, and clinical applications particularly highlighting emerging applications in biomedical imaging.



1. INTRODUCTION

Vesicular stomatitis virus (VSV) is an enveloped virus that belongs to the Rhabdoviridae family. Vulnerable hosts for VSV include cattle, horses, and pigs. The negative-sense single-stranded RNA genome of the virus encodes for five proteins found in the gene order: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and large protein (L)[1] (Figure 1). The simplicity of its genomic organization and the ease of growing the virus to high titers in most mammalian cells have propelled VSV as a workhorse for the understanding of myriad aspects of cellular and viral processes. The VSV glycoprotein VSV-G is the determinant for viral attachment and entry into susceptible host cells. The VSV-G contains ∼500 amino acids and is post-translationally modified with N-linked glycosylation and fatty acid addition. In the matured virion, the protein remains in trimeric and fusogenic form. This form of the protein enables viral attachment to the host cell receptors and promotes virus entry via a clathrin-mediated endocytic mechanism. Continued research in molecular virology, viral protein structure, host responses to viral infections and viral pathogenesis, etc. has resulted in a detailed understanding of the biochemical and biophysical properties of VSV-G protein and its associated functions. Additionally, the ease of production, purification, and customization processes has made the VSV-G-based platform an attractive tool for various biomedical applications.

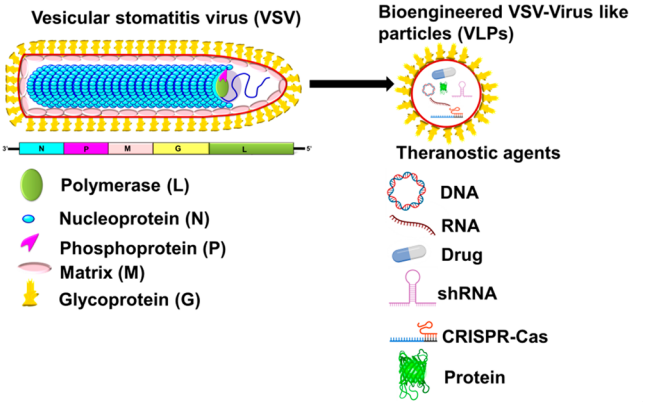


Figure 1. Schematic of wild-type VSV and VSV-G-based nanocarrier. The bullet-shaped VSV genome encodes five proteins: polymerase (L), nucleoprotein (N), phosphoprotein (P), matrix protein (M), and glycoprotein (G). It serves as a platform for bioengineering from VSV-G for different biological applications.

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Although VSV has been exploited to understand many important aspects of cellular, molecular, and viral processes, two unique aspects of the biology of VSV have led to significant advances in studies on many other viruses. The early observations that VSV incorporating the envelope proteins of other viruses can be readily generated from cells coinfected with the viruses[2] firmly established the concept of "pseudotypes" proposed previously.[3] With the ability to manipulate the VSV genome to generate recombinant viruses lacking the viral G protein, pseudotype VSVs incorporating envelope proteins of different families of viruses have been generated in recent years. These studies have led to better understanding of the entry mechanisms of viruses, identifying receptors, generating live viral vaccine vectors, developing virus-neutralization tests, and identifying neutralizing antibodies, as well as developing pseudotype viruses for studies under low-containment facilities for viruses that otherwise require high-containment facilities. Additionally, the use of VSV-G protein in pseudotyping to generate stable, high titers of lentiviral vectors with broad cell tropism[4] has revolutionized the field of gene and cell therapy.

The other unique aspect of VSV-G protein is its ability to generate virus-like particles (VLPs) when ectopically expressed in a variety of eukaryotic cells. These VLPs contain the viral G protein on the envelope and can incorporate cytoplasmic contents of cells from which these VLPs are produced. These properties have been exploited to produce VLPs that can package proteins, therapeutic molecules, large macromolecular complexes such as CRISPR-Cas9 ribonucleoprotein complexes, and naked DNA.[5] In addition, VSV-G has been modified to target to specific cell types. Interestingly, when VSV-G protein was expressed from an autonomously replicating viral replicon lacking the viral structural proteins, the G protein packaged the replicon and generated novel infectious particles that could be propagated in cultured cells.[6] Overall, these studies point to the importance of VSV-G for its use in diverse areas of biomedical research and applications. In this manuscript, we highlight the recent development of VSV-G platforms and their biomedical applications, particularly emphasizing its potential for biomedical imaging.

2. STRUCTURE AND FUNCTION OF VSV-G

VSV-G is a type I transmembrane protein folded into a structure with a short carboxy-terminal cytoplasmic tail and transmembrane domain and an amino-terminal ectodomain representing the bulk of the protein. It exists in a dynamic equilibrium between monomeric and trimeric forms both on the viral envelope and in infected cells; however, the biologically active form of the protein is considered to be trimeric.[7] The cytoplasmic domain of VSV-G interacts with the M protein for efficient virus assembly, although modifications of this domain including deletions and replacements with those from other viral and nonviral sequences can lead to viral assembly. The ectodomain is responsible for binding to the host cell receptor and subsequent fusion with the endosomal membrane for the uncoating of the viral genome. It also contains antibody recognition sites as well as viral neutralizing epitopes.

VSV-G is a class III fusion protein[8] that is characterized by its pH-dependent reversible conformational changes between pre- and postfusion states.[9−12] This pH-dependent reversible conformational transition is thought to be critical for its intracellular transport in acidic compartments of the Golgi apparatus in an inactive form to the cell surface where it exists in an active fusogenic form.[15] The crystal structures of both pre-and post-fusion postfusion forms of VSV-G have been resolved.[17] The protein's monomer is made up of four distinct domains. Domain I of the VSV-G is dense with β sheets, whereas domain II is centrally located and involved in protein trimerization. Domain IV is the fusogenic domain that was discovered to be inserted into the loop of the pleckstrin homology domain III.[13] VSV-G binds to host cell receptors such as low-density lipoprotein (LDL) and its family members. During binding with the host cell receptor, the protein undergoes significant rearrangement and mediates membrane fusion. VSV-G remains as a trimer in the mature virion at pH 7 in the prefusion state.[14] The large changes in the protein's orientation are caused by the secondary structure, a hinge region found between the pleckstrin homology domains and the fusion domain. This rearrangement causes the protein to transition from the prefusion to the postfusion state. The conformational transition in VSV-G caused by low pH allows the viral and endosomal membranes to fuse, resulting in the release of the viral genome into the cytosol where the ribonucleoprotein complex initiates transcription and subsequent translation of viral proteins including VSV-G. The nascent protein is cotranslationally inserted into membranes of the endoplasmic reticulum (ER). The ectodomain of VSV-G undergoes post-translational modifications (PTM) in the form of N-glycosylation at two asparagine residues. Following PTM, the VSV-G forms microdomains at the plasma membrane that serve as sites for virion assembly and budding. The reversible pH-dependent conformational changes with fusogenic properties and signature sequences in its various domains make VSV-G a unique molecular tool for researchers.

3. BIOMEDICAL APPLICATIONS OF THE VSV-G

3.1. Development of the VSV-G VLPs. Viruses are naturally evolved nanocarriers. They efficiently transport their genomes to specific host cells. Because a virus is an obligate intracellular parasite, carrying the genome to the host cell is quintessential for its replication. Most viruses have a lipid membrane (envelope) derived from the host cell; however, some viruses are nonenveloped, with no membrane but only the proteinaceous capsid. While protecting the genome, the capsids or envelopes have the ability for conformational changes to unlock and swiftly transfer the genome to the permissible cells. The viral envelope typically consists of a lipid bilayer with transmembrane glycoproteins embedded in it. In the case of VSV and many other negative-sense (NS) RNA virus infections, mainly three forms of particles are produced in the infected cells. In the majority of cases, the budding particles carrying a full-length copy of the viral genome are mature infectious virions. Another form, namely, defective interfering (DI) particles, have a truncated genome package.

These defective genomes have packaging signal sequences but are deficient for self-replication as they do not contain the necessary genes expressing viral polymerase functions. However, these particles are structurally homologous to the parent virus but need a helper virus for their genome replication. The third category of particles is completely empty in nature; these are self-assembled protein cage-like structures lacking nucleic acid in their core. These are also structural homologues of the parent virus and are often termed virus-like particles or VLPs. The nature of protein-protein interactions dictates the assembling of the viral protein

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Table 1. Biomedical Applications of VSV-G VLPs

tools	short description	in vivo/in vitro
plasmid-based gene therapy	coexpression of VSV-G and IL-12 for gene therapy	in vitro and in vivo
viral vector pseudotypes	flaviviruses, coronaviruses, paramyxoviruses, herpes simplex virus, and other lentiviral vectors are the gold standard; however, VSV-G lentivirus has been shown to be effective	in vivo
delivery of mRNA	VSV-G VLPs as mRNA-delivery vehicle	in vitro
delivery of CRISPR-Cas9	VSV-G-based envelope carrier	in vivo
selective delivery of protein and nucleic acids	VSV-G-established VLPs as a delivery vehicle	in vitro
exogenous protein delivery	protein transduction method based on VSV-G-based nanovesicles	in vitro and in vivo
cancer immunotherapy	VSV-G expresses macrophages acting as fusogenic cells to deliver the GOI	in vitro and in vivo
functionalization of drug-delivery vectors	layer-by-layer (LbL) microcarrier surface functionalized by VSV-G to enhance cellular uptake	in vivo

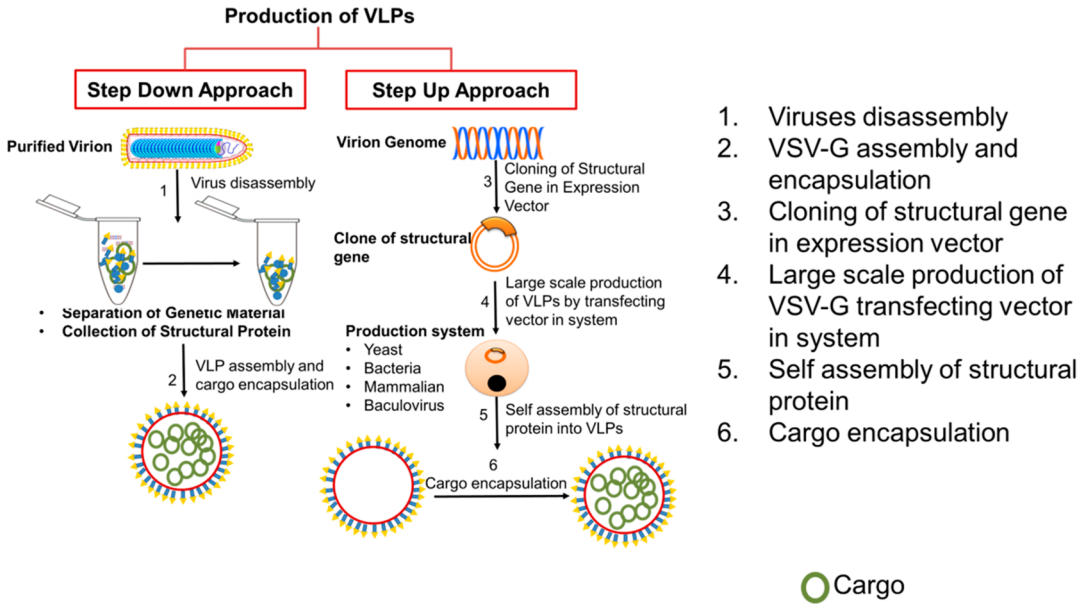

Figure 2. Schematic representation of step-up and step-down approaches for the production, purification, and cargo loading in a VSV-G nanocarrier. Step-up approach: in this process, the gene of a viral structural protein is cloned in an expression vector for protein production on a large scale. However, the step-down approach begins with using the parent virus by depleting its genetic material. In the end, the protein is collected as particles with no infectious properties. The downstream process of purification, such as clarification, purification, and polishing, is used to obtain the purified protein.

subunits that form the VLPs. These structures naturally remain hollow inside and therefore can carry specific cargo volumes. Like the parent virion, VLPs could be either enveloped or just have a proteinaceous capsid layer.

As mentioned earlier, the VSV-G protein has the natural ability to assemble into VLPs when expressed in cells. Investigators have optimized the production of VLPs based on recombinant expression of G in numerous cell types and functionalized these for various biological and medical applications. As the VSV glycoprotein's structural transitions are reversible, this property has allowed researchers to introduce a package-specific volume of cargo/small molecules into the VLPs, resulting in useful reagents. On the basis of these principles, VSV-G-expressing cell lines are extensively used as a packaging system for the production of important biological reagents such as gene delivery platforms, gene-editing tools, mRNAs, etc.

When expressed in human or mammalian cells, VSV-G self-assembles and is released at a very high titer in the form of lipid-bound fusogenic nanovesicles. Several molecular tools based on VSV-G are successfully utilized for clinical research and aptly used to package biological materials, including small-molecule drugs, protein, peptides, DNA, mRNA, etc., as shown in Table 1. Like the parent virions, these VSV-G-derived carrier nanoparticles (NPs) undergo the same pathway for their attachment and internalization. During endocytosis, the conventional NP formulation encounters lysosomal hydrolase, resulting in the degradation of most of the delivery agents. On the other hand, the VSV-G-encapsulated formulation confers endolysosomal escape to deliver cargo into the cytoplasm of target cells. Besides these, VSV-G VLPs have excellent biophysical properties that enable their use at the forefront as a nanocarrier. These include stable morphology, well-known surface properties, ease of large production, a higher surface area-to-mass ratio, and the ability to encapsulate small molecules.

Additionally, VSV-G particles are often compatible with a wide variety of active pharmacological moieties. Because a

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surface-exposed amino acid residue is amenable to further modifications and tagging, these particles could therefore be further modified with specific ligands and/or moieties for cell receptor targeting and site-specific delivery of packaged formulation. Hence, a smaller quantity of drugs is required when administered through VLPs, eliminating overdose and offsite target-related toxic effects. Often, this could result in cost-effective treatment. Thus, with desired modifications, these nanovesicles have been shown to transport eukaryotic genes, mRNA, proteins, and organelles to different mammalian cells and tissues. Because a VSV-G VLP is functionally active like that of the parent virion, it has emerged as a potential drug-delivery platform in recent years.

3.1.1. Methods of VSV-G VLP Production. VLPs are highly organized, multiprotein hollow structures resembling that of a natural virion. On the basis of the physical properties of the parent virus, two significant modes are used for VLP production: (i) in the step-down approach, the genome is depleted from the parent virus and the VPLs are assembled, and (ii) in the step-up approach, there is an expression of required viral proteins in a suitable host and subsequent assembly of VLPs (Figure 2). In the latter case, expressed viral proteins self-assemble into particles. Various expression systems, including prokaryotic, eukaryotic, and cell-free systems, have been explored for VLP production. However, a fair understanding of the viral structural components is essential for the selection of an appropriate expression system. The popular expression systems include bacteria, yeast, plant, insect, and mammalian cells.

Although the bacterial system is the most commonly used recombinant protein expression platform, several limiting factors such as lack of or improper PTM, contamination of endotoxins, incomplete disulfide-bond formation, and protein solubility restrict the use of a bacterial expression system for VLP production of enveloped viruses, including VSV-G. Similarly, yeast expression systems, such as those of *Saccharomyces cerevisiae* and *Pichia pastoris*, are not yet optimized for VSV-G VLP production. Although genome manipulation of these hosts has resulted in improved PTM of the desired protein, issues like protein trafficking, proper protein folding, etc. restrict the use of the yeast system for VSV-G VLP expression.

The insect cell expression system is widely used for generating viral proteins on laboratory and industrial scales. The significant advantage of this system includes PTM of the protein, like mammalian system capability of fast and large-scale cultivations, high protein yield, etc. Typically, this system utilizes recombinant baculovirus as a gene expression tool. Here, the coexpression of multiple proteins by designing polycistronic recombinant baculovirus can also be achieved. However, the most commonly used system is the mammalian cell expression system, where cell lines such as Chinese hamster ovary (CHO) cells, baby hamster kidney-21 (BHK-21), CEVEC's amniocyte production cell line derived from human amniocytes, human embryonic kidney 293T (HEK293T), Vero cells, etc. are routinely used. However, low protein yield, higher production cost, long-expression time, and the possibility to carry infection with other pathogens such as bacteria, fungi, or viruses are potential drawbacks of mammalian cell expression systems for generating clinical-grade VLPs. Apart from the suitability of the platform, one of the important criteria for achieving a high titer VLP production is the spontaneous release of VLPs from the mammalian cell membrane. Often, eukaryotic cells pose additional challenges in the form of host restriction factors that interfere with virus release, which is an innate mechanism to limit viral spread. However, VSV-G can antagonize interferon (IFN)-inducible antiviral host cell factor tetherin (BST-2, CD317) and enhance the release of VLPs. Thus, VSV-G can be produced in high titer even in the presence of tetherin-expressing cells.[15]

3.2. Emergence of VSV-G as an Accessory Tool for Gene Therapy. Due to its broad cell tropism, VSV-G supports efficient gene transfer into a wide range of target cells such as neurons, bone marrow stem cells (BMSCs), rat insulinoma cell line INS-1, etc. For the past three decades, VSV-G-based, pseudotyping platforms to package retroviral/lentiviral vectors are being used for stable gene transfer in eukaryotic cells.[4] The emergence of a VSV-G-based retroviral/lentiviral packaging platform made significant improvements against murine leukemia virus-derived vectors in two major aspects: (i) removal of the barrier of the restricted host-cell range and (ii) production of high titer with uniformly infectious particles. As a result, this platform revolutionized gene therapy model studies in suitable animal hosts. Subsequently, a plethora of basic research was completed prior to any clinical trial studies for gene therapy. Progress in this direction led to associated technology for surface modification of VLPs in the form of maneuvering amino acid residues or by tagging anchor moiety to enhance gene delivery efficiency. Further, VSV-G can form complexes with plasmid DNA, creating fusogenic structures. These complexes show enhanced transfection properties for gene delivery without a lipofection reagent.[5] Interestingly, the engineering of the VSV-G delivery system has been demonstrated to improve nucleic acid transduction efficiency. The mRNA of a particular gene is preferred because it is transient and does not pose any risk of integration in target cells, so appropriate delivery systems are being tested continuously. In this context, researchers have demonstrated VSV-G-based chimeric viral particles for the delivery and transduction of green fluorescence protein (GFP) mRNA in monocytes and pluripotent stem cells (iPS cells).[16] Similarly, targeted delivery of small VSV-G anchored extracellular vesicles showed superior gene transduction efficiency. Experiments conducted by Liu et al. demonstrated that siRNA targeting programmed death-1 (PD-L1) can also be efficiently administered in vivo in CT26 tumor-bearing mice using a VSV-G-based system.[17] This approach enabled robust silencing of PDL-1 in tumor tissue, which in turn elevated effector $CD8^+$ T cell function, resulting in faster tumor clearance. Because of these excellent examples, there is a growing interest in gene delivery using the VSV-G-based system for a variety of biomedical applications.

3.3. VSV-G-Encapsulated Nanoformulations for Biomedical Imaging. Biomedical imaging, mainly to detect the early stages of cancer, is in high demand for timely therapeutic intervention. Exogenously administered contrast agents are routinely used for in vivo studies for mapping the contour of the developing tumor. In this context, modified viruses are being demonstrated for the delivery of exogenous contrast agents to aid available imaging techniques such as positron–electron tomography (PET), magnetic resonance imaging (MRI), and optical imaging. The success of the technique rests on surface modification with appropriate ligands such as metal nanoparticles and contrast agents. The use of VSV-G-based nanoparticles has recently been demonstrated as an improved imaging carrier.[18] Currently, fluorescence imaging has emerged

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as an effective noninvasive technique for cancer diagnosis in preclinical settings. However, it is difficult to look into the human body beyond penetration depth (1 mm) using visible radiation. However, biological tissues are transparent in the near-infrared (NIR) range; thus, deeply buried inhomogeneity can be observed by NIR imaging. NIR imaging offers the advantage of minimal autofluorescence and enhanced tissue-penetration depth (5−10 mm).[19] Also, the inclusions of exogenous contrast agents in the NIR spectrum offer an improved signal-to-noise ratio and a better contrast for easy diagnosis. In this context, we recently demonstrated that fabricated NIR-active VSV-G VLPs encapsulating indocyanine green (ICG) dye, a U.S. Food and Drug Administration (FDA) approved contrast agent, could be used in routine biomedical imaging. Here VSV-G VLPs were generated in the mammalian expression system (HEK 293T cells) in the secretory form and were purified from the conditioned media using simple ultracentrifugation steps. These VLPs encapsulated ICG, which we termed NIR active viral nanoconstructs (NAVNs). Compared to the free form of ICG, the VSV-G packaged form not only enhanced the photostability of this contrast agent but also significantly improved the targeted delivery of the contrast agent into the cytoplasm. The NIR imaging showed enhanced fluorescence intensity and improvement in the signal-to-noise ratio (SNR) of NAVN-treated cells compared to free ICG. Even after 72 h of treatment with cells, the encapsulated form was ∼60% optically active while the free ICG activity fell below ∼20%.[18] This approach also showed dramatic improvement in the circulating half-life of ICG in preclinical mouse model studies. The improved circulating half-life of ICG was partly because of the stability of the formulation in blood circulation aided by sustained release of the drug from VLPs over time. In vivo experiments conducted in BALB/c by our group demonstrated that free ICG was almost eliminated from the circulation within 24 h of administration, while encapsulated ICG remained beyond 72 h of administration (Figure 3). For this study, the NAVNs and free ICG solution were administered intravenously via tail vein in BALB/c mice. The free ICG was given at a dose of 3 mg/kg of body weight. Fluorescence images were taken 24 and 48 h after injection. These images were captured at an excitation wavelength of 745 nm, emission wavelength of 840 nm, and exposure time of 2−6 s. Histopathological examination of these mice did not show any significant cytotoxicity effect when administered with VSV-G VLPs, thus showing the biocompatibility of these particles in a preclinical setup. Therefore, as a proof of concept, we demonstrate that bioengineered VSV-G encapsulating ICG could be a superior NIR-imaging platform and has the potential for immediate medical-imaging applications.

3.3.1. Mechanistic Insight into VSV-G ICG Interaction. To better understand the mechanism of ICG packaging, we performed a molecular dynamics (MD) simulation to check the interaction of ICG with VSV-G protein. MD simulations are frequently preferred to investigate the structure and dynamics of biomolecules and various time-dependent dynamical processes. The MD simulation approach is well-established and suitable for elucidating the ligand-binding function, predicting binding poses, and determining the conformational dynamics of the binding pockets. Herein, we looked at the interactions between ICG and VSV-G by employing molecular docking and MD simulation in conjunction with the free energy calculation. The dye molecule

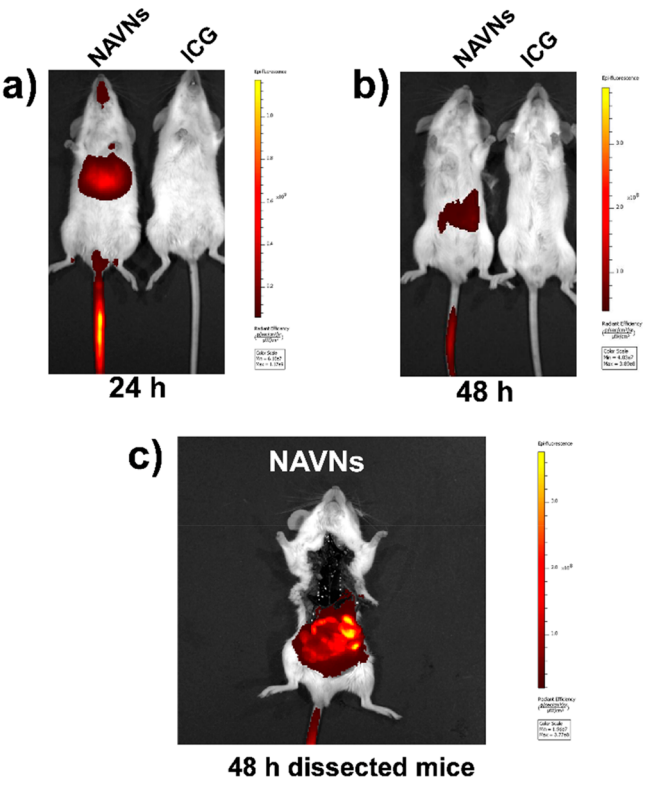

Figure 3. In vivo wholebody NIR imaging of the biodistribution of free ICG and NAVNs injected intravenously in BALB/c mice. (a) NIR fluorescence image of mice 24 h postinjection of ICG and NAVNs. (b) NIR fluorescence image of mice 48 h postinjection of ICG and NAVNs. (c) NIR fluorescence image of mice dissected 48 h postinjection of NAVNs. The figure is part of the doctoral research work carried out by Suman Bishnoi.

(ICG) was docked into the binding pocket of VSV-G (PDB: 5I2S) using the Schrödinger suite. The best pose from the docking study was subjected to MD simulation in both neutral and acidic (pH 5.0) aqueous solutions by performing conventional molecular dynamics (cMD) and constant pH molecular dynamics (CpHMD) using the AMBER18 suite. For cMD, before the production run, two-step minimization followed by a stepwise heating step was performed. In the case of CpHMD, after every 200 fs of explicit MD simulation, the protonation change event was attempted for all possible residues in the GB implicit model. Details of our simulation protocol can be found in our previous studies.[20] In the current study, we performed MD simulations for 100 ns in both the neutral and acidic conditions, which stabilize after 55 ns, as shown in Figure 4a.

We also investigated the stability of the ICG molecule in the binding pocket by estimating the center of the mass distance of the ligand and the binding site residues throughout the simulation time (Figure 4b). In the case of the neutral condition, ICG was found to remain tightly bound to the binding pocket (Figure 4c). On the other hand, in the acidic condition (pH 5), ICG deviated from its original binding site and remained attached to the protein's surface (Figure 4d). This implies that, in the acidic environment, the ligand binding is destabilized.

This analysis is further complemented by estimating the binding free energy of the VSV-G/ICG complex using the molecular mechanics/Poisson−Boltzmann surface area (MM/

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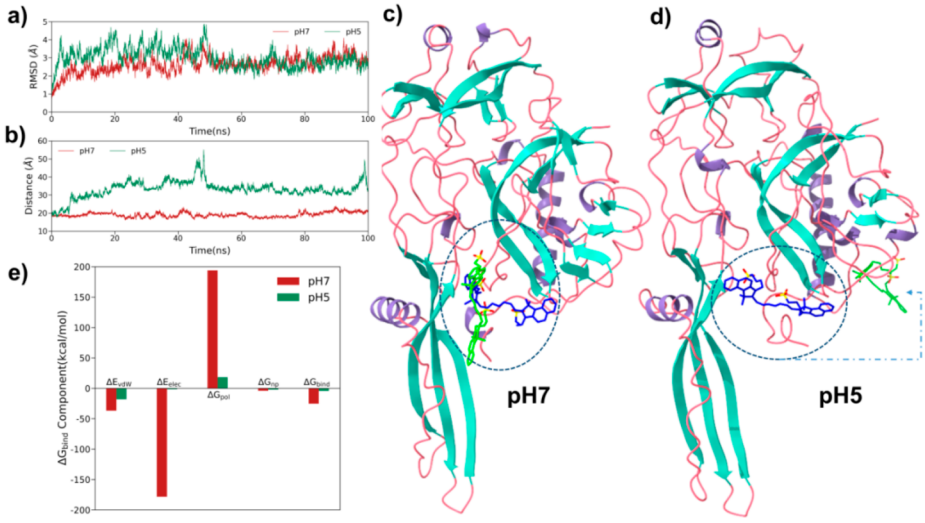

Figure 4. MD simulations of the VSV-G/ICG complex in neutral (pH 7.0, red) and acidic (pH 5, green) conditions. (a) Time evolution of root-mean-squared deviation (RMSD) of backbone atoms of VSV-G. (b) Time evolution of the center of mass (CoM) distance between the protein and ICG. (c) Superimposition of the initial conformation with the final structure obtained from MD simulation at pH 7.0. The initial and final coordinates of ICG are depicted in blue and green. (d) Superimposition of the initial snapshot with the final structure obtained from the MD simulation at pH 5, where the initial and final coordinates of ICG are in blue and green. (e) Various components of total binding free energy (kcal/mol) of ICG in neutral and acidic conditions.

PBSA) scheme. The estimated binding free energy is −25.16 kcal/mol in the neutral solution, almost 6 times more favorable than the acidic state (−4.41 kcal/mol). The binding affinity of ICG in low pH decreased significantly due to the loss of profitable contribution from the intermolecular van der Waals and electrostatic interactions (Figure 4e). Overall, the binding free energy calculations corroborate the center of mass distance analysis.

Thus, the molecular dynamics simulation study showed that VSV-G undergoes conformational changes in a low pH environment and triggers the release of ICG to the cytoplasm of the target cell. Further, the VSV-G−ICG interaction enhanced the half-life of ICG in the cells, thereby improving the physical and optical stability of the contrast agent. Thus, VSV-G could further be customized and engineered by fusing NIR fluorescent protein or an antibody for in vivo imaging.[21]

3.4. Therapeutic Agent Delivery. Pharmaceutical agents can be encapsulated in a VSV-G nanocarrier to enhance delivery potential; for example, the partially purified VSV-G in the form of vesicles released from a transfected cell is commonly used to encapsulate various therapeutic agents. This system can encapsulate a diverse array of small molecules including protein, RNA, siRNA, and ICG, including the delivery of genome editing tools. In this context, the VSV-G platform is explored for delivering the CRISPR-SpCas9-ribonucleoprotein complexes (RNPs) to various transformed, pluripotent stem cell cardiomyocytes in an in vivo model.[22] VSV-G has also been demonstrated to package recombinant proteins by cotransfection of VSV-G with Gag-GFP for direct delivery into the cytoplasm of target cells.[23] Furthermore, these nanocarriers could efficiently incorporate proteins, such as tetracycline repressor (TetR) trans activator and murine cationic amino acid transporter-1 (mCAT-1), and the receptor of murine leukemia virus (MLV) envelope to target cells.[24] Thus, this method of protein transduction is a versatile tool for the transport of various cytoplasmic, nuclear surface proteins into the target cells.

4. SITE-DIRECTED MODIFICATION OF VSV-G FOR ENHANCED DELIVERY

VSV-derived VLPs are structurally and functionally homologous to their parent virions. The crystal structure of VSV-G provides detailed information related to various domains and its associated functions, including protein-protein interaction for multimerization, exposed residues for immune targeting, binding and fusion loop for host cell receptors, etc. The crystal structure further reveals the presence of a transmembrane domain, which undergoes structural rearrangement in response to low pH. Various modifications of VSV-G have been carried out at the amino acid level to enhance the targeting and cargo-delivery abilities of the nanoconstructs. Researchers have identified that VSV-G has several feasible insertion sites between amino acids 1 and 400, which could tolerate 9-amino acid cyclin-RGD (cRGD) peptides. Insertion of 49-amino acid echistatin (snake venom disintegrin) peptide binding between amino acids 351 and 352 in the middle of the lateral domain of VSV-G specifically targeted integrins expressed on the tumor tissue.[25] On the basis of this evidence, it is possible to customize VSV-G with an appropriate modification of amino acid sequences to improve tissue-specific targeting of these nanocarriers. At the postproduction stage, these nanocarriers can also be modified by insertion or chemical conjugation of targeting moieties at the surface of the particles.[16]

Besides this, VSV-G bears a fusion-loop structure and a hydrophobic part, potentially exposed to interact with host cellular organelles. The structural analysis predicts the possibility of modifying individual domains of VSV-G, keeping its self-assembling ability intact. Previous studies have confirmed that VSV-G can be genetically engineered for tissue targeting by incorporating collagen-binding decapeptide N-WREPGRMELN-C showing specific attachment with the collagen matrix.[26] Likewise, the single-chain antibody fragment (scFv) of 253-amino acid peptide has also been successfully incorporated within VSV-G for specific binding with MHC-I.[27] All of these type of modifications make VSV-G an attractive and amenable candidate for VLP generation.

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5. IMMUNOGENICITY AND NEUROTOXICITY ISSUES AND POSSIBLE MITIGATION STRATEGY

Despite significant research progress, the clinical application of VSV-G platforms for drug delivery and biomedical imaging is limited. This is primarily due to a strong humoral (B cell) response against VSV-G protein. The protein has several immunodominant epitopes, including the most potent one at the C-terminal end of the protein. Thus, VSV-G VLPs can induce a strong humoral immune response even after a single dose of application. The host-neutralizing antibodies (IgG) are primarily targeted at the G protein. This strong immunogenicity leads to serum inactivation of the VSV-G-based nanocarrier, making this platform unsuitable for multiple applications. Importantly, like other rhabdoviruses, VSV-G could also elicit life-long memory T cell and B cell responses. This is a major hindrance to multidose therapeutic applications of VSV-G-derived reagents, including the VLPs mentioned here. The other drawback associated with the VSV-G-based application is the inherent neurotropism associated with G.[28] Although this attribute could be exploited for broader organ-specific applications, including delivery to the brain, adequate modifications and amino acid alternations are desired for such application. Unlike in a VSV-infected host, where G is produced at a logarithmically higher scale associated with virus replication, biomedical application of VLPs is limited in quantity and scale and organ-specific. As a result, the chances of neurotoxicity are minimized to tolerable limits. Deleting the specific immunodominant epitope or alterations of a few amino acids could thus avoid immunogenicity to a large extent. Thus, the combination of epitope deletion and site-directed mutagenesis could make an immune-tolerable G.[29] Moreover, the immunodominant region of VSV-G can be switched with the serum-resistant glycoprotein protein of other viruses.[30] We hope that, with these strategies, both neurotoxicity and immunogenicity issues could be addressed to a larger extent. The other approach for addressing immunogenicity relies on switching the glycoproteins from another serotype. VSV is prevalent in two major serotypes, VSV Indiana (VSV_I) and VSV New Jersey (VSV_{NJ}), having neutralizing antibody responses that are not cross-reactive. These differential immune response properties thus could be exploited for repeat applications of a VSV-G-derived nanocarrier. Besides this approach, the glycoprotein of other Vesiculoviruses, such as the Chandipura virus (CHPV), could be explored in combination with VSV_I and VSV_{NJ} for multiple in vivo applications.

6. CONCLUSIONS

A protein-based nanocarrier holds promise for a variety of biomedical applications, including efficient drug-delivery platforms, biomedical imaging, gene-editing tool delivery, etc. Remarkable advancements in molecular biology and nanotechnology centering on viral proteins have created new approaches to delivering active pharmaceutical agents in living systems. Many such products have entered clinical trials. Still, this approach faces challenges. Viral proteins offer a versatile platform ranging from vaccine development and gene therapy to drug-delivery applications. Due to the well-studied and unique properties associated with VSV-G, we have achieved pinpoint cell-specific targeting of pharmacological agents. A few properties of the VSV-G, such as effective cell penetration in the absence of endosomal sequestration, multivalence, and biocompatibility, make this platform a valuable research tool. As discussed, VSV-G nanocarriers can encapsulate many active molecules; still, there is room for improvement. One major drawback lies in the immune response to G protein, preventing multiple applications in the same host. However, epitope manipulation and change of serotypes are postulated to tackle such issues. Finally, we anticipate that continued future research in this direction would make the VSV-G platform a viable option for biomedical applications in clinical settings.

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S.R., S.B., and H.R. wrote the manuscript. S.R., R.R., and A.K. contributed to figures and tables. P.K., S.G., and D.N. conceptualized the work and corrected the manuscript. A.K.P. contributed the structure and functions discussion part of the manuscript and the editing of the manuscript.

Notes

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